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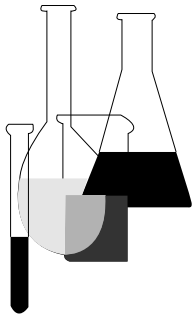
Health Effects Test Guidelines

OPPTS 870.5250
Gene Mutation in
Neurospora crassa



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

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OPPTS 870.5250 Gene mutation in Neurospora crassa.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are OPPT 40 CFR 798.5250 Gene mutation in Neurospora crassa and OPP 84–2 Mutagenicity Testing (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982.

(b) **Purpose**. *Neurospora crassa* (*N. crassa*) is a eukaryotic fungus which has been developed to detect and study a variety of genetic phenomena including chemically induced mutagenesis. *N. crassa* can be used to detect both forward and reverse gene mutation. These mutations are detected by biochemical or morphological changes in the treated population. The most commonly used mutation assay in *N. crassa* measures forward mutation in the *ad-3* region of the genome.

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definition also applies to this test guideline.

Forward mutation is a gene mutation from the wild (parent) type to the mutant condition.

(d) **Reference substances.** These may include, but need not be limited to, ethyl or methyl methanesulfonate.

(e) **Test method**—(1) **Principle.** The detection of forward mutations at the *ad-3* locus in either homokaryons or heterokaryons may be used. However, use of two component heterokaryons is recommended because of the greater range of mutations which can be recovered. In either case, the test relies on the identification of purple (mutant) colonies among a large number of white (wild-type) colonies. A representative sample of purple colonies can be recovered and thoroughly analyzed genetically.

(2) **Description.** Forward mutations at the *ad-3* locus can be detected using noncolonial strains of *N. crassa* grown on media containing sorbose as well as glucose. Under these conditions, colonies are formed and reproducible colonial morphology results. Adenine-requiring mutants which accumulate a reddish-purple pigment can be readily identified and counted.

(3) **Strain selection**—(i) **Designation.** At the present time, heterokaryon 12 is recommended for use in this assay. The use of other strains may also be appropriate.

(ii) **Preparation and storage.** Stock culture preparation and storage, growth requirements, method of strain identification, and demonstration

of appropriate phenotypic requirements should be performed using good microbiological techniques and should be documented.

(iii) **Media.** Frie's No. 3 minimal medium or Westgaard's Synthetic medium with 1.5 percent agar or any medium known to support growth and characteristic colonial morphology may be used in the assay.

(4) **Preparation of conidia.** Stock cultures should be grown on minimal medium to select for single colonies with noncolonial morphology. Single-colony isolates then should be inoculated into agar flasks and incubated at 35 °C for 48 hours to select colonies with spreading growth patterns in which mycelia cover the entire flask. Flasks should be incubated at 23-25°C and those with bright orange conidia selected for preparation of conidial suspensions. Suspensions should be diluted for use in distilled water.

(5) **Metabolic activation.** Conidia should be exposed to a test substance both in the presence and absence of an appropriate metabolic activation system.

(6) **Control groups.** Concurrent positive and negative (untreated and/or vehicle) controls both with and without metabolic activation should be included in each experiment.

(7) **Test chemicals**—(i) **Vehicle.** Test chemicals and positive control reference substances should be dissolved in an appropriate vehicle and then further diluted in vehicle for use in the assay.

(ii) **Exposure concentrations.** (A) The test should initially be performed over a broad range of concentrations selected on the basis of a preliminary assay. Effective treatment times should also be selected in the preliminary assay.

(B) Among the criteria to be taken into consideration for determining the upper limits of test chemical concentration are cytotoxicity and solubility. Cytotoxicity of the test chemical may be altered in the presence of metabolic activation systems. For toxic chemicals, the highest concentration tested should not reduce survival below 10 percent of that seen in the control cultures. Relatively insoluble chemicals should be tested up to the limits of solubility. For freely soluble nontoxic chemicals, the upper test chemical concentration should be determined on a case-by-case basis.

(C) Each test should include five treatment points; two at fixed concentrations for different time periods and three at varying concentrations for fixed periods of time.

(D) When appropriate, a positive response should be confirmed by testing over a narrow range of concentrations.

(f) **Test performance**—(1) **Treatment.** (i) Growing or nongrowing conidia should be exposed to the test chemical with and without metabolic activation. At the end of the exposure period, treatment should be terminated by chemical quenching. The quenching solution may contain 0.1 percent sodium thiosulfate.

(ii) Conidia should then be plated on the appropriate media to determine mutation induction and viability. At the end of the incubation period, colonies should be scored for viability and mutation induction.

(iii) Mutants should be classified according to color and morphology.

(iv) Both mutation frequency and viability should be determined both immediately before and immediately after chemical treatment.

(2) **Incubation conditions.** All plates in a given test should be incubated for the same time period. This incubation period may be from 2 to 7 days at 30°C.

(3) **Number of cultures.** Generally, 15 to 20 individual plates per concentration should be used.

(g) **Data and report**—(1) **Treatment of results.** Individual plate counts for test substance and controls should be presented for both mutation induction and survival. The mean number of colonies per plate and standard deviation should be presented. Data should be presented in tabular form indicating, as applicable, numbers of colonies counted, numbers of mutants identified, and classification of mutants (e.g., color segregants). Sufficient detail should be provided for verification of survival and mutation frequencies.

(2) **Statistical evaluation.** Data should be evaluated by appropriate statistical techniques.

(3) **Interpretation of results.** (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of mutant colonies. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of mutant colonies or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) **Test evaluation.** (i) Positive results from the *ad-3* system in *N. crassa* indicate that, under the test conditions, the test substance causes mutations in the DNA of this organism.

(ii) Negative results indicate that under the test conditions the test substance is not mutagenic in *N. crassa*.

(5) **Test report.** In addition to the reporting recommendations as specified under 40 CFR part 792, subpart J, the following specific information should be reported:

(i) Strain of organism used in the assay.

(ii) Test chemical vehicle, doses used, and rationale for dose selection.

(iii) Method used for preparation of conidia.

(iv) Treatment conditions, including length of exposure and method used to stop treatment.

(v) Incubation times and temperature.

(vi) Details of both the protocol used to prepare the metabolic activation system and of its use in the assay.

(vii) Dose-response relationship, if applicable.

(h) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Brockman, H.E. and de Serres, F.J. Induction of *ad-3* mutants of *Neurospora crassa* by 2-aminopurine. *Genetics* 48: 597–604 (1963).

(2) de Serres, F.J. and Malling, H.V.Measurement of recessive lethal damage over the entire genome and at two specific loci in the ad-3 region of a two-component heterokaryon of *Neurospora crassa*. Chemical mutagens: principles and methods for their detection. Vol. 2, Ed. Hollaender, A. Plenum, New York and London (1971) pp. 311–342.

(3) Matzinger, P.K. and Ong, T–M. In vitro activation of aflatoxin B_1 to metabolites mutagenic in *Neurospora crassa. Mutation Research* 37:27–32 (1976).